Ca\textsuperscript{2+} exchange with troponin C and cardiac muscle dynamics

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Controversy abounds in the cardiac muscle literature over the rate-limiting steps of cardiac muscle contraction and relaxation. However, the idea of a single biochemical mechanism being the all-inclusive rate-limiting step for cardiac muscle contraction and relaxation may be oversimplified. There is ample evidence that Ca\textsuperscript{2+} concentration and dynamics, intrinsic cross-bridge properties, and even troponin C (TnC) Ca\textsuperscript{2+} binding and dissociation can all modulate the mechanical events of cardiac muscle contraction and relaxation. However, TnC has generally been thought to play no role in influencing cardiac muscle dynamics due to the idea that Ca\textsuperscript{2+} exchange with TnC is very rapid. This definitely is the case for isolated TnC, but not for the more sophisticated biochemical systems of reconstituted thin filaments and myofibrils. This review will discuss the biochemical influences on Ca\textsuperscript{2+} exchange with TnC and their physiological implications.

KEYWORDS
Troponin; Cardiac; Muscle; Myosin; Calcium

1. Introduction

Discovered by Ebashi over 40 years ago (for review\textsuperscript{1}), troponin is a hetero-trimeric complex consisting of three subunits: TnC (troponin C, the Ca\textsuperscript{2+}-binding subunit), TnI (the inhibitory subunit), and TnT [the tropomyosin (Tm)-binding subunit] (for review\textsuperscript{2}). Multiple protein–protein interactions between TnI, TnT, actin, and Tm anchor the Tn complex into the thin filament (for review\textsuperscript{2}). Ca\textsuperscript{2+} binding to TnC leads to a series of conformational rearrangements in Tn and Tm, which reveals strong myosin binding sites on actin, allowing cross-bridges to cycle, and muscle contraction. Relaxation occurs as intracellular Ca\textsuperscript{2+} declines, Ca\textsuperscript{2+} dissociates from TnC, Tm blocks strong myosin-binding sites on actin, and cross-bridges detach (for review\textsuperscript{2}).

Although Ca\textsuperscript{2+} must bind to TnC to allow contraction, and dissociate from TnC to allow relaxation, the contribution of TnC to the kinetics of contraction and relaxation is controversial. The heart works to meet the metabolic demands of the body. Since the body’s demands can fluctuate widely from rest to intense exercise, the heart relies on the ability of the molecular machinery within each heart muscle cell to keep pace. These cellular processes can become impaired in many forms of heart disease.\textsuperscript{6–8} Thus, it is essential to understand the fundamental elements that contribute to the rates of contraction and relaxation so that better treatments and prevention can be devised for systolic and diastolic dysfunction, of which TnC is a prime target.\textsuperscript{9}

2. Troponin C structure and function

TnC is a member of the EF-hand super-family of Ca\textsuperscript{2+}-binding proteins and consists of N- and C-terminal globular domains connected by a central \(\alpha\)-helix. Each domain contains a pair of helix–loop–helix EF-hand metal-binding motifs (for review\textsuperscript{2,10}). Although all four EF-hands of skeletal TnC are capable of binding Ca\textsuperscript{2+} ions, the first EF-hand of cardiac TnC is apparently unable to bind Ca\textsuperscript{2+} due to several loop residue substitutions.\textsuperscript{11} As a result, the second EF-hand is the only functional Ca\textsuperscript{2+}-binding site in the N-terminal domain of TnC. It is generally accepted that the second EF-hand of TnC regulates muscle contraction, whereas the third and fourth EF-hands play a structural role by anchoring TnC into the Tn complex. Although these are generally accepted paradigms for the separate function of the two domains of TnC, the C-terminal domain of TnC...
may play more than just a structural role in cardiac muscle contraction/relaxation.12

Many studies have focused on elucidating the interactions between TnC and TnI/TnT. Earlier studies determined that TnC and TnI interact in anti-parallel orientation, with the N-terminal domain of TnC interacting with the C-terminal domain of TnI.13 The recently determined crystal structure of the core domain of the Tn complex provides a deeper insight into the interactions between TnC and TnI/TnT.14 The core domain of the Tn complex can be divided into two subdomains, the regulatory head and the IT arm (Figure 1), connected by a flexible linker. The regulatory head consists of the N-terminal domain of TnC and the switch region of TnI. The switch region is situated between the two putative actin-binding segments of TnI and forms multiple van der Waals interactions with the hydrophobic patch on the surface of the Ca2+-bound N-terminal domain of TnC. According to the ‘drag and release’ mechanism proposed for the regulation of muscle contraction and relaxation (for review15,16), Ca2+ induces the movement of the switch region towards the hydrophobic patch on the N-terminal domain of TnC, dragging along the adjacent actin-binding segments of TnI, causing their release from actin and ultimately force generation. Eventually, a decline in intracellular [Ca2+] would lead to the dissociation of the switch region of TnI from the hydrophobic patch on the N-terminal domain of TnC, resulting in muscle relaxation (for review15,16).

Numerous studies have also focused on elucidating the factors controlling metal- and protein-binding properties of TnC in biochemical systems of increasing complexity (Figure 2). A lot of important information on the conformational changes occurring in TnC upon Ca2+ or ligand binding has been obtained from spectroscopic studies such as circular dichroism, NMR, and X-ray crystallography.14,17–19 In addition, researchers have utilized either intrinsic (primarily utilized only for isolated TnC20,21) or extrinsic fluorescent probes as tools to obtain a more complete understanding of how TnC activates and deactivates the thin filament in a Ca2+-dependent manner.22–26 It is clear that there are many biochemical, physiological, and pathophysiological factors that can influence the Ca2+ sensitivity of force development and specifically of the Tn complex (for review3,8,16,27). For instance, TnI is phosphorylated during β-adrenergic stimulation.28 The activation of protein kinase A increases the phosphorylation state of TnI (and other EC-coupling and sarcomeric proteins) and has been associated with accelerated relaxation of the heart (for review16,27). In addition, it is also becoming clear that activation of another family of kinases, protein kinase C, can either enhance or decrease the Ca2+ sensitivity of force development through differential phosphorylation of a number of Ser and Thr residues on TnI.29,30 In addition, a host of assorted naturally occurring Tn subunit mutations linked to various cardiomyopathies can enhance or decrease the Ca2+ sensitivity of the thin filament through currently unknown mechanisms that ultimately alter cardiac performance and morphology.22,23

3. Measurements of Ca2+ exchange kinetics with troponin C

It has been generally assumed that rates of Ca2+ exchange with TnC in muscle are more rapid than the kinetics of contraction and relaxation. Indeed, the rates of Ca2+ dissociation from the second EF-hand of TnC, measured in the stopped-flow apparatus utilizing the fluorescence of IAANS attached to TnC on Cys56 or Trp fluorescence of TnCF27W, were at least an order of magnitude faster than the mechanical event of relaxation.21,31 The very rapid rate of Ca2+ dissociation from the second EF-hand of TnC was also measured directly following changes in the spectroscopically sensitive Ca2+ chelators Quin-2 or Bapta (700–800 s−1 at 4°C22) and utilizing NMR spectroscopy (5000 s−1 at 30°C). In addition, the rate of Ca2+ association to the
second EF-hand of TnC, measured by the stopped-flow technique, was also reported to be extremely rapid, yet not diffusion controlled, contrary to dogma. However, the rates of Ca$^{2+}$ exchange with TnC in muscle are unlikely to be the same as with isolated TnC, since TnC functions not in isolation but as a part of the thin filament-bound Tn complex.

The Ca$^{2+}$-binding properties of TnC are drastically modified upon incorporation into the Tn complex. In fact, binding of Tnl/TnT increases the Ca$^{2+}$ affinity of the second EF-hand of TnC by at least an order of magnitude. This increase in the Ca$^{2+}$ affinity is largely due to a decreased rate of Ca$^{2+}$ dissociation, which was measured in a number of studies by the stopped-flow technique utilizing fluorescently labelled TnC (for review). Also measured following the fluorescence of Quin-2 in a stopped-flow apparatus, the rate of Ca$^{2+}$ dissociation from the second EF-hand decreases from $>1000$ s$^{-1}$ in isolated TnC to $\sim120$ s$^{-1}$ upon binding of Tnl, and further to $\sim35$ s$^{-1}$ upon binding of TnT at 15°C. Incorporation of the Tn complex into the reconstituted thin filament (actin–Tm–Tn) desensitizes the regulatory N-terminal domain of TnC to Ca$^{2+}$ (see Kobayashi and Solaro and Davis et al.) and accelerates the rate of Ca$^{2+}$ dissociation to $\sim105$ s$^{-1}$ (see Davis et al.) (Figure 3A). Consistent with the effects of myosin binding to actin and sensitizing the thin filament to Ca$^{2+}$ in cardiac muscle, addition of myosin S1 reverses the thin filament-induced desensitization, even though myosin does not directly bind to Tn. Addition of myosin S1 to the thin filament also slows Ca$^{2+}$ dissociation from the second EF-hand of TnC to 13 s$^{-1}$, a rate comparable with that of cardiac muscle relaxation (5–11 s$^{-1}$ at 15°C) (Figure 3B). Recently, the rate of Ca$^{2+}$ dissociation from the regulatory domain of fluorescently labelled TnC in isolated myofibrils was measured using stopped-flow technology in the presence of ATP to activate cycling cross-bridges (although the strongly bound states under these conditions of no load may be very short lived) at 20°C, which occurred at $\sim39$ s$^{-1}$. Since the rate of Ca$^{2+}$ dissociation has not been measured in intact cardiac muscle, it may be that the reconstituted thin filament system and possibly the isolated myofibrils are the minimal biochemical systems required to adequately address the physiological rate of Ca$^{2+}$ dissociation from TnC.

The mechanisms behind the changes in the Ca$^{2+}$ sensitivity and exchange rates with the second EF-hand of TnC occurring upon incorporation of Tn into the thin filament are unclear. The 'drag and release' mechanism proposed for the regulation of muscle contraction implies that TnC and actin compete for the binding of TnI. The competition for the regulation of muscle contraction implies that TnC are unclear. The 'drag and release' mechanism proposed occurring upon incorporation of Tn into the thin filament system and possibly the isolated myofibrils can directly or indirectly affect the rates of Ca$^{2+}$ dissociation from the second EF-hand of TnC.

The affinity of TnC for Ca$^{2+}$ can vary depending on the species of origin. For instance, the affinity of trout TnC for Ca$^{2+}$ is increased compared with that of mammalian TnC, due to the several residue substitutions in the regulatory N-terminal domain. Pharmacological compounds, such as bepridil, have been shown to affect the rate of Ca$^{2+}$ dissociation from the second EF-hand of TnC and modify its Ca$^{2+}$-binding sensitivity. However, the effects of bepridil or species-dependent mutations on TnC have not been measured in the more sophisticated biochemical systems. All the major thin filament proteins (Tnl, TnT, Tm, and actin) can directly or indirectly affect the Ca$^{2+}$ sensitivity and the kinetics of Ca$^{2+}$ exchange with TnC. A number of studies have demonstrated that Tn subunit isofrom switching, phosphorylation of Tnl, and mutations in Tn subunits linked to cardiomyopathies can sensitize or desensitize TnC to Ca$^{2+}$ and/or affect the rates of Ca$^{2+}$ exchange with TnC. For instance, slow skeletal Tnl (ssTnl; expressed in embryonic and early postnatal hearts and possibly

Figure 3 Effects of actin–tropomyosin and myosin S1 on Ca$^{2+}$ dissociation from troponin. (A) Incorporation of troponin into the thin filament (actin–Tm) increases the rate of Ca$^{2+}$ dissociation from $\sim42$ to $\sim105$ s$^{-1}$. (B) Addition of myosin S1 to the reconstituted thin filament (actin–Tm–Tn) slows the rate of Ca$^{2+}$ dissociation to $\sim13$ s$^{-1}$. Monocysteine troponin C was labelled with IAANS on engineered Cys 53. Data were adapted from Davis et al.
re-expressed during disease\textsuperscript{46-47} increases the Ca\textsuperscript{2+} affinity and decreases the rate of Ca\textsuperscript{2+} dissociation from the second EF-hand of TnC in the Tn complex, on the thin filament, and in the presence of myosin S1\textsuperscript{44}. The rate of Ca\textsuperscript{2+} dissociation from the second EF-hand of TnC in the Tn complex was dependent on the TnT isoform present.\textsuperscript{48} It has been known for a long time that phosphorylation of Ser 23 and 24 of TnI by PKA (in response to β-adrenergic stimulation of the heart) decreases the Ca\textsuperscript{2+} affinity of the Tn complex and increases the rate of Ca\textsuperscript{2+} dissociation, which appears to still occur on the thin filament.\textsuperscript{26,49} The effect of mutation on the Ca\textsuperscript{2+} affinity of TnC can vary depending on the biochemical system studied. For instance, mutations of TnI linked to hypertrophic or restricted cardiomyopathy (R145G, R145W, D190H, and R192H) increased the Ca\textsuperscript{2+} sensitivities of the Tn complex on the thin filament, but not in isolation.\textsuperscript{23} Thus, it should not be assumed that the behavior of the simple biochemical systems (TnC and Tn) will always reflect the more sophisticated biochemical systems or intact muscle.

4. Rate-limiting step(s) of muscle contraction/relaxation

Just as there are different levels of complexity to study the kinetics of Ca\textsuperscript{2+} exchange with TnC, there are many physiological systems to study the kinetics of cardiac muscle contraction and relaxation ranging from in vivo whole-heart function to isolated, single myofibrils. Tn subunit mutation, phosphorylation, and isoform variation have been implicated in affecting the contractility and kinetics of cardiac muscle contraction in vivo.\textsuperscript{8,47,50} These experiments suggest that the Ca\textsuperscript{2+}-binding properties of Tn in the cardiac muscle cell are among many modulators of cardiac muscle contractility. However, it is not a simple matter to directly correlate a Tn modification to altered cardiac physiology. For instance, the heart has additional compensatory mechanisms that can also go awry or stabilize the new physiological set point potentially caused by the Tn modification, such as adjusting the Ca\textsuperscript{2+} transient, kinase/phosphatase activity, and up/down regulating other proteins.\textsuperscript{47,51-53}

Perhaps the most direct technique to measure of the effect of TnC or Tn subunit modification on cardiac muscle performance is the ‘skinned’ muscle preparation (for review\textsuperscript{3,4}). In intact muscle preparations, the rise of intracellular Ca\textsuperscript{2+} from the second EF-hand of TnC in the Tn complex was dependent on the TnT isoform present.\textsuperscript{48} It has been known for a long time that phosphorylation of Ser 23 and 24 of TnI by PKA (in response to β-adrenergic stimulation of the heart) decreases the Ca\textsuperscript{2+} affinity of the Tn complex and increases the rate of Ca\textsuperscript{2+} dissociation, which appears to still occur on the thin filament.\textsuperscript{26,49} The effect of mutation on the Ca\textsuperscript{2+} affinity of TnC can vary depending on the biochemical system studied. For instance, mutations of TnI linked to hypertrophic or restricted cardiomyopathy (R145G, R145W, D190H, and R192H) increased the Ca\textsuperscript{2+} sensitivities of the Tn complex on the thin filament, but not in isolation.\textsuperscript{23} Thus, it should not be assumed that the behavior of the simple biochemical systems (TnC and Tn) will always reflect the more sophisticated biochemical systems or intact muscle.

4.1 Influences on the rate of cardiac muscle contraction

At the cellular level, contraction of cardiac muscle involves multiple steps: (i) concentration of intracellular Ca\textsuperscript{2+} elevates; (ii) Ca\textsuperscript{2+} binds to the second EF-hand of TnC; (iii) the N-terminal domain of TnC binds to the C-terminal domain of TnI; (iv) TnI dissociates from actin; (v) Tm shifts its position on actin; (vi) weakly bound cross-bridges transition to strongly bound cross-bridges further moving Tm on actin; and (vii) force and/or muscle shortening occurs (for review\textsuperscript{3,4}). In intact muscle preparations, the rate of intracellular Ca\textsuperscript{2+} significantly precedes force development and is not considered to be rate-limiting for contraction (for review\textsuperscript{4}). In addition, Bell et al.\textsuperscript{52} recently demonstrated that the Ca\textsuperscript{2+}-dependent structural transitions in TnC also precede force development. Furthermore, it is generally believed that steps 3 through 5 are rapid, allowing step 6 to be rate-limiting for contraction.\textsuperscript{63,64} It is also generally assumed that the rate of transition from weakly bound cross-bridges to strongly bound cross-bridges is primarily determined by the kinetics of myosin.\textsuperscript{63,64} However, the concept of a single process being the all-inclusive rate-limiting step for cardiac muscle contraction might be oversimplified.\textsuperscript{65} It could be that the rate of transition from weak to strong cross-bridge binding is not only influenced by the characteristics of myosin, but also by the Ca\textsuperscript{2+}-binding properties of TnC on the thin filament, as well as the level of free Ca\textsuperscript{2+}.

There are two contractile isozymes of the myosin heavy chain in cardiac muscle, α and β, at varying ratios depending on the state of health and species.\textsuperscript{66} Supporting the notion that the intrinsic properties of myosin dictate the rate of contraction, α-myosin goes through the ATPase cycle faster than β-myosin, and likewise, cardiac muscle containing primarily α-myosin contracts faster than muscle containing primarily β-myosin.\textsuperscript{59,60,67} In addition, the products of ATP hydrolysis, inorganic phosphate (P\textsubscript{i}) and adenosine diphosphate (ADP), increase and decrease the maximal rate of myosin ATPase activity, respectively (for review\textsuperscript{4}). Again, in agreement with myosin controlling the rate of cardiac muscle contraction, increases in P\textsubscript{i} accelerate the rate of cardiac muscle contraction.\textsuperscript{60,68} The myosin isozymes and ATP byproducts modulate the rate of cardiac muscle...
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contraction independently of TnC. However, it is well established that the rate of cardiac muscle contraction is also strongly Ca\(^{2+}\) dependent (for review\(^3\)). In skinned cardiac muscle studies, the rate of contraction increases \(\sim\) 3 to 5-fold with increasing Ca\(^{2+}\) concentration, reaching a maximal rate when force is maximal (for review\(^69\)). It would appear that none of the steps in the cross-bridge cycle are directly modulated by Ca\(^{2+}\) (for review\(^3\)). As discussed earlier, TnC is the Ca\(^{2+}\) sensor for muscle contraction. Even though the rate of Ca\(^{2+}\) binding to TnC is not diffusion controlled,\(^{21}\) the regulatory domain of TnC still binds Ca\(^{2+}\) significantly faster than the rate of cardiac muscle contraction.\(^{62}\) Thus, the rate of Ca\(^{2+}\) binding to TnC itself is not rate-limiting for contraction. However, it may be that as Ca\(^{2+}\) rises, TnC binds the Ca\(^{2+}\) rapidly and either concomitantly or very quickly thereafter activates the thin filament via the proposed drag and release mechanism to a state dependent on the amount of Ca\(^{2+}\). The thin filament is now primed to recruit weakly bound cross-bridges to strongly bound cross-bridges to generate force. The higher the free Ca\(^{2+}\) (up to a saturating amount), the more strongly bound cross-bridges can interact with actin and the faster the force develops. If this is true, then the rate of cardiac muscle contraction at submaximal concentrations of Ca\(^{2+}\) should be modulated by the Ca\(^{2+}\) sensitivity of TnC.

Recently, two TnC mutants were designed that increase or decrease the Ca\(^{2+}\) sensitivity of cardiac muscle force development.\(^{21,68}\) The two TnCs did not significantly alter the minimal or maximal \(k_r\) of rat’s skinned trabeculae but did increase or decrease \(k_s\) at submaximal Ca\(^{2+}\) concentrations in the presence and absence of \(P_i\) (Figure 4).\(^{68}\) Thus, it was concluded that the intrinsic properties of both myosin and TnC can affect the rate of cardiac muscle contraction. That the effects of the TnC mutants were most prevalent at subsaturating Ca\(^{2+}\) may be of physiological consequence considering cardiac muscle functions primarily at subsaturating Ca\(^{2+}\) levels, and many of the cardiac disease-associated Tn mutations alter the Ca\(^{2+}\) sensitivity of force development (for review\(^4\)). Alternatively, it has been suggested that the Ca\(^{2+}\) sensitivity of TnC plays no role in the rate of muscle contraction, since the \(k_r\) vs. relative force relationship was similar for control and different conditions that sensitize TnC and myofibrils to Ca\(^{2+}\).\(^{60,70}\) However, in order to match levels of relative force production, different concentrations of Ca\(^{2+}\) had to be used under the different conditions, and thus the Ca\(^{2+}\)-sensitizing techniques must have accelerated a TnC that does not bind Ca\(^{2+}\) at the regulatory domain and thus does not support force development, maximal force production by the muscle decreases, but maximal \(k_r\) remains unaffected.\(^{58}\) These studies suggest that relative force and \(k_r\) can be dissociated, and that as long as a minimal region of the thin filament is maximally activated by Ca\(^{2+}\) through the remaining endogenous TnC, \(k_r\) remains maximal.

4.2 Influences on the rate of cardiac muscle relaxation

Relaxation is as critical as contraction for a normal beating heart. Impaired relaxation occurs in approximately half of heart failure patients (for review\(^71\)). Cardiac muscle relaxation is a complex process involving the reversal of the seven biochemical steps listed earlier for contraction (not necessarily in the same order) as well as mechanical inhomogeneities of the sarcomeres.\(^{41}\) The decline in the intracellular Ca\(^{2+}\) transient, dissociation of Ca\(^{2+}\) from the regulatory domain of TnC, and cross-bridge detachment have all been hypothesized as rate-limiting steps for relaxation (for review\(^3,63,72\)). There is no general agreement on the relative contribution of each of these steps to the rate of mechanical relaxation. Similar to the process of contraction, the mechanism of relaxation may not be governed by a single rate-limiting step but may involve all three processes working together.

Much of the work that supports the fall in Ca\(^{2+}\) as being rate-limiting for relaxation comes from isolated cardiac myocytes (for review\(^2\)). These experiments have added a vast wealth of information to the cardiac field by simultaneously measuring the time course of the Ca\(^{2+}\) transient, with changes in contraction and relaxation (indicated by unloaded cell shortening and re-lengthening). The use of myocytes from transgenic mice has most clearly demonstrated a direct correlation between the fall of the Ca\(^{2+}\) transient and relaxation kinetics (for review\(^73\)). For instance, overexpression of SERCA or knockout of the SERCA regulatory protein phospholamban significantly accelerates both Ca\(^{2+}\) sequestration and re-lengthening. On the other hand, poisoning SERCA depresses both Ca\(^{2+}\) sequestration and relaxation.\(^{74}\) These experiments indicate that the fall in Ca\(^{2+}\), which is controlled primarily by SERCA, may be rate-limiting for relaxation. However, these studies were conducted with unloaded myocytes. Increasing the load on the myocytes slows the time course of relaxation independent of the Ca\(^{2+}\) transient.\(^{75}\) Even though the rate of the Ca\(^{2+}\) transient decline is probably not rate-limiting for loaded relaxation, its duration can modulate the time course of

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![Figure 4](https://example.com/figure4.png)

**Figure 4** Effects of Ca\(^{2+}\) sensitizing and desensitizing troponin C mutants on \(k_r\) in rat’s skinned trabeculae. The figure shows that sensitizing or desensitizing troponin C to Ca\(^{2+}\) increased or decreased, respectively, \(k_r\) at submaximal Ca\(^{2+}\). All troponin C mutants contained the F27W mutation. Data were adapted from Norman et al.\(^{68}\) The inset shows the crystal structure of the regulatory N-terminal domain of troponin C from the Ca\(^{2+}\)-saturated troponin core domain (PDB file 1J1E\(^4\)), with Val 44 shown in red, and Phe 20 shown in blue. The inset was generated using Rasmol.\(^{88}\)
relaxation. For instance, increased expression of SERCA or the soluble Ca\(^{2+}\)-buffering protein parvalbumin in vitro accelerates Ca\(^{2+}\) removal and increases cardiac muscle relaxation in vivo.\(^76\),\(^77\)

At room and body temperatures, the rate-limiting step of isometric relaxation of intact rat trabeculae appears to be determined by the myofilaments.\(^78\),\(^79\) For instance, stretching cardiac muscle increases the number of available strongly bound cross-bridges and slows relaxation without changing the Ca\(^{2+}\) transient (for review\(^79\)). This effect of slowed relaxation was also observed in ventricular isovolumic contractions and in ventricles of conscious dogs (for review\(^80\)). One interpretation of these results is that a feedback mechanism of strongly bound cross-bridges increases the subsequent strong binding of other cross-bridges, causing relaxation to be prolonged. In support of this hypothesis, relaxation was drastically impaired when the strongly bound cross-bridge analogue, NEM-S1, was introduced into rat’s skinned ventricular myofibrils.\(^40\)

More direct effects of cross-bridge kinetics on relaxation can be determined in skinned cardiac preparations induced to relax by rapid Ca\(^{2+}\) sequestration by the photo-labile Ca\(^{2+}\) chelator diozo-2.\(^81\),\(^82\) For instance, increasing the concentration of ADP decreases cross-bridge detachment and the rate of relaxation, whereas increasing the concentration of Pi increases cross-bridge detachment and the rate of relaxation.\(^81\),\(^82\) Similarly, relaxation is accelerated by Pi in small bundles of cardiac myofibrils induced to relax by removal of Ca\(^{2+}\) by rapid solution exchange.\(^83\) Thus, intracellular metabolites known to alter the kinetics of cross-bridge detachment modify the rate of cardiac muscle relaxation independent of the fall in Ca\(^{2+}\). In addition, guinea pig and human adults primarily express the slower \(\alpha\)-myosin isozone, whereas adult mice and rats primarily express the faster \(\beta\)-myosin isozone in their ventricles.\(^83\),\(^84\) However, hypothyroidism in rats shifts myosin expression to nearly 100% \(\beta\)-myosin without any changes in Tn.\(^83\)

Skinned cardiac muscles form the same or different species expressing \(\alpha\)-myosin relax two to five times faster than those expressing \(\beta\)-myosin.\(^38\),\(^58\)–\(^60\) Thus, changing myosin heavy chain expression also modulates the rate of cardiac muscle relaxation. The conclusion from some of these studies is that myosin kinetics alone are rate-limiting for relaxation, based on the assumption that Ca\(^{2+}\) dissociation from TnC is extremely rapid (for review\(^61\)), which is true for isolated TnC, but may not be the case with the more physiologically relevant biochemical model systems (Figure 3).\(^24\) In any regard, there is no doubt that the intrinsic characteristics of myosin play a fundamental role in regulating the rate of cardiac muscle relaxation.

The evidence suggesting that the Ca\(^{2+}\) exchange kinetics with TnC may also influence the rate of cardiac muscle relaxation comes from studies of TnI modification. For instance, ssTnI increases the Ca\(^{2+}\) affinity and decreases the rate of Ca\(^{2+}\) dissociation from the Tn complex in isolation, on the thin filament, and in the presence of myosin S1,\(^24\) consistent with delayed myocyte relaxation and in vivo diastolic dysfunction in transgenic mice expressing ssTnI in the heart.\(^47\) Furthermore, it has long been known that \(\beta\)-adrenergic stimulation of the heart accelerates the rate of cardiac muscle relaxation and is associated with increasing the rate of Ca\(^{2+}\) dissociation from TnC through PKA phosphorylation of TnI.\(^28\),\(^84\) In addition, truncation of TnI associated with cardiac ischaemia, or a familial hypertrophic cardiomyopathy-related TnI mutation, increased the Ca\(^{2+}\) sensitivity of force development and delayed the rate of relaxation of isolated cardiac myofibrils.\(^55\),\(^56\) However, the Ca\(^{2+}\)-sensitizing compound bepridil had no effect on the rate of relaxation of isolated cardiac myofibrils, but did sensitize the force developed by the myofibrils to Ca\(^{2+}\).\(^60\) It is currently unknown how bepridil or any of the disease-associated Tn modifications affect TnC kinetics in the more sophisticated biochemical systems, or for that matter in muscle, although it is typically assumed only the Ca\(^{2+}\) dissociation rate is altered. However, it has been proposed that ssTnI may affect the Ca\(^{2+}\) association rate to a greater extent than the rate of Ca\(^{2+}\) dissociation from TnC on the thin filament in the presence of myosin S1.\(^24\) Since the rate of Ca\(^{2+}\) dissociation from TnC has not been measured in muscle or force-bearing myofibrils, it is difficult to know with certainty whether the rate of Ca\(^{2+}\) dissociation from TnC is too rapid or slow enough to influence relaxation. However, there is circumstantial evidence that TnC is still bound with Ca\(^{2+}\) during relaxation on the basis of ‘extra’ Ca\(^{2+}\) being released from the myofilaments late in relaxation and after rapid release of force during relaxation in intact trabeculae (see Jiang et al.\(^78\) and references within). This extra Ca\(^{2+}\) is thought to be released from TnC as cross-bridges are rapidly detached, which in turn decreases the Ca\(^{2+}\) sensitivity of Tn by accelerating the rate of Ca\(^{2+}\) dissociation from TnC.\(^24\) Thus, it may be that Ca\(^{2+}\) dissociation from TnC and cross-bridge kinetics work together to modulate the rate of cardiac muscle relaxation at the level of the myofilaments. The effects of TnC on relaxation could be determined by utilizing TnC mutants that alter the rate of Ca\(^{2+}\) dissociation from TnC in the complex biochemical systems.

5. Conclusions

In summary, controversy abounds in the cardiac muscle literature over the rate-limiting steps of cardiac muscle contraction and relaxation. The idea of cardiac muscle contraction/relaxation being controlled by a single biochemical step may be oversimplified. For instance, as argued earlier, cardiac muscle contraction/relaxation can be modulated by Ca\(^{2+}\) levels/kinetics, Ca\(^{2+}\) association/dissociation from the regulatory binding site of TnC, and cross-bridge kinetics. Thus, Ca\(^{2+}\) exchange with TnC may be one of a number of mechanisms in the arsenal of cardiac muscle that can be modulated to alter cardiac muscle performance in health and disease.

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